New loci associated with kidney function and chronic kidney disease

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Multiple New Loci Associated with Kidney Function and Chronic Kidney Disease: The CKDGen consortium

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Abstract

Chronic kidney disease (CKD) is a significant public health problem, and recent genetic studies have identified common CKD susceptibility variants. The CKDGen consortium performed a meta-analysis of genome-wide association data in 67,093 Caucasian individuals from 20 population-based studies to identify new susceptibility loci for reduced renal function, estimated by serum creatinine (eGFRcrea), cystatin C (eGFRcys), and CKD (eGFRcrea <60 ml/min/1.73m^2; n = 5,807 CKD cases). Follow-up of the 23 genome-wide significant loci ($p<5\times10^{-8}$) in 22,982 replication samples identified 13 novel loci for renal function and CKD (in or near *LASS2, GCKR, ALMS1, TFDP2, DAB2, SLC34A1, VEGFA, PRKAG2, PIP5K1B, ATXN2, DACH1, UBE2Q2*, and *SLC7A9*) and 7 creatinine production and secretion loci (*CPS1, SLC22A2, TMEM60, WDR37, SLC6A13, WDR72, BCAS3*). These results further our understanding of biologic mechanisms of kidney function by identifying loci potentially influencing nephrogenesis, podocyte function, angiogenesis, solute transport, and metabolic functions of the kidney.

Keywords

genome-wide association; renal disease; population-based; genetics; chronic kidney disease

Introduction

Chronic kidney disease (CKD) is estimated to affect over 13% of adults1 and is increasing in prevalence.1;2 This poses a significant global disease burden as the risk for end stage renal disease (ESRD), cardiovascular morbidity, and mortality increases with declining glomerular filtration rate (GFR),3 the most commonly used measure of kidney function. In addition, CKD incurs substantial expenditures in the US,4 with similar trends expected globally.5

Despite the increasing prevalence of CKD, our understanding of the underlying risk factors and pathophysiologic mechanisms remains incomplete.5 Hypertension and diabetes are major risk factors for CKD.6 However, the marked variability in the development of CKD in the setting of hypertension and diabetes demonstrates that additional underlying factors contribute to its etiology.[7] In particular, studies have consistently demonstrated important genetic contributions to estimated GFR (eGFR), CKD and ESRD.[8;9] Using genome-wide association, we have recently identified susceptibility variants for renal function and CKD at the *UMOD*, *SHROOM3*, and *STC1* loci in nearly 20,000 individuals.[10] Together, single nucleotide polymorphisms (SNPs) at these loci explain only 0.43% of the variance in eGFR, [10] suggesting that additional loci remain to be identified.

Thus, we have now performed a genome-wide association meta-analysis in 67,093 Caucasian participants from 20 general population-based cohorts within the CKDGen consortium, followed by independent replication of our findings in 22,982 Caucasian individuals. We analyzed GFR estimated from serum creatinine by the Modification of Diet in Renal Disease (MDRD) Study equation (eGFRcrea) as well as CKD (eGFRcrea <60 ml/min/1.73m^2). To discriminate true susceptibility loci for renal function from those related to creatinine production and secretion, we used GFR estimated from a second serum marker of kidney function, cystatin C (eGFRcys).

RESULTS

Study Samples

Overall, 90,075 individuals (67,093 in Stage 1 discovery and 22,982 Stage 2 replication) contributed information to the analysis of eGFRcrea, 84,740 individuals (62,237 Stage 1 discovery and 22,503 Stage 2 replication) to the analysis of CKD, and 26,071 to the analysis of eGFRcys (20,957 Stage 1 discovery and 5,114 Stage 2 replication; Table 1).

Meta-Analysis in CKDGen Stage 1 Discovery Cohorts

Table 2 summarizes information for the 28 genomic loci that contained at least one genome-wide significant SNP association ($p<5\times10^{-8}$) for any of the three discovery traits; the SNP with the lowest p-value at each locus is presented. In addition to confirming 5 known loci,[10] we identified 23 novel loci containing genome-wide significant SNPs (p-values between 4.5×10^{-8} and 3.8×10^{-12}): 20 SNPs were identified in association with eGFRcrea, 2 SNPs with CKD, and 1 SNP with eGFRcys. Of note, rs7805747 in the *PRKAG2* gene was identified in discovery analyses for both eGFRcrea and CKD, as was the known lead SNP at the *UMOD* locus.

Figure 1A shows the genome-wide $-\log_{10}$ p-value plot from Stage 1 discovery association analyses with eGFRcrea, Figure 1B with CKD, and Figure 1C shows the eGFRcys results. The respective quantile-quantile plots are presented in Supplementary Figure 1. Study-specific and median imputation quality for the lead SNPs can be found in Supplementary Table 2.

Corroborating Evidence with eGFRcys

Because serum creatinine concentration is influenced both by renal function as well as by creatinine production or secretion, we used eGFRcys as a second measure of renal function to help distinguish between true renal function loci and creatinine production or secretion loci. Thus, of the 23 newly discovered loci, 16 were classified as renal function loci based on a direction-consistent association with eGFRcys with p-value of <0.05 (Table 2 and Supplementary Table 3), and 7 were classified as loci related to creatinine metabolism. One SNP, rs653178 at the *ATXN2* locus, was identified primarily in association with eGFRcys.

Stage 2: Independent Replication of Genome-wide Significant Discovery Findings

The lead SNP at each of the 20 novel loci for eGFRcrea, the 2 loci for CKD, and the novel locus for eGFRcys were evaluated for independent replication in Stage 2 analyses with the respective discovery trait. After meta-analysis of Stage 1 discovery and Stage 2 replication results, the associations for all but 3 SNPs (rs16864170 at *SOX11*, rs1933182 at *SYPL2*, rs4014195 at *RNASEH2C*) became more significant (Table 2). Additionally, 16 of these 20 SNPs also showed a significant association in the replication samples alone (one-sided p-value<0.0025 for eGFRcrea, p<0.025 for CKD, and p<0.05 for eGFRcys, Table 2). Thus, after integrating evidence for replication with association results for eGFRcys, 13 replicated loci for renal function and 7 replicated loci likely related to creatinine metabolism were

identified. Of note, rs653178 at the *ATXN2* locus, which was identified primarily in association with eGFRcys, was also associated with eGFRcrea in the combined discovery and replication results. Regional association plots for all replicated loci related to renal function are shown in Supplementary Figure 2.

Of the SNPs that were validated by Stage 2 replication and were also associated with eGFRcys, *ALMS1*, *DAB2*, *SLC34A1*, *PRKAG2*, *VEGFA*, *DACH1*, and *SLC7A9* can be linked to renal function and/or disease and are highlighted together with *LASS2* and *GCKR* in Box 1. The remaining novel renal function loci were located in or close to *TFDP2*, *PIP5K1B*, *UBE2Q2*, *and ATXN2*. In spite of a lack of clear biological connection to renal function at these loci, findings were consistent across eGFRcrea, CKD, and eGFRcys analyses. More information on these genes is presented in Supplementary Box 1.

Of the 20 SNPs that replicated, the remaining 7 SNPs were not associated with eGFRcys and hence were considered as likely creatinine production or secretion loci (*CPS1*, *SLC22A2*, *TMEM60*, *WDR37*, *SLC6A13*, *WDR72*, and *BCAS3*). More information on these loci is presented in Supplementary Box 1.

SNPs associated with eGFRcrea are also associated with CKD

The majority of the 13 validated renal function loci were nominally associated with CKD (Table 3), underscoring how the use of intermediate phenotypes can provide insight into disease-based traits. The odds ratios associated with CKD for each additional copy of the minor allele ranged from 0.93 to 1.19, and minor allele frequencies ranged from 0.20 to 0.50.

Stratified Analyses and Measures of Clinical Relevance

Since diabetes mellitus and hypertension are major risk factors for kidney disease, we investigated the association of the replicated renal function loci with eGFRcrea stratified by diabetes or hypertension status in the discovery cohorts. None of the SNPs reported in Table 2 differed significantly across strata of diabetes and hypertension ($p<0.008$, Bonferroni-corrected alpha of 0.1 for 13 tests).

The 13 confirmed and the three previously identified renal function loci account for 1.4% of the variation in eGFRcrea. A genetic risk score was computed using all 16 validated renal loci (13 novel, 3 known) and data from the ARIC study. Across categories of the genetic risk score, mean eGFRcrea ranged from 86.9 (SD 18.7) to 71.1 (SD 14.7) in individuals in the lowest (10) to the highest risk score category (25), and CKD prevalence ranged from 3.9 to 23.6%, respectively.

Analyses of Expression-Associated SNPs (eSNPs)

To obtain evidence for the presence of functional variants at the identified genomic risk loci and to prioritize genes in the associated regions, we focused on SNPs previously identified in genome-wide studies as significantly related to gene expression in liver (n= 3,322),11 lymphocytes (n=29,094) 12 or lymphoblastoid cell lines (n=10,823).13 These expression SNPs (eSNPs) were then evaluated for their association with eGFRcrea, CKD, and eGFRcys from the discovery analysis. Table 4 shows that 9 of the 20 novel susceptibility loci identified for eGFRcrea (7/13 renal function loci) contained one or more significant eSNPs in one or more of the expression tissues queried. In addition, three of the previously identified loci (*SHROOM3*, *GATM*, and *CST3*) also contained at least one eSNP. The correlation (r^2) between the significant eSNP at each locus with the strongest LD to lead SNP ranged from 0.01 (*FBXO22*) to >0.9 (*DAB2*, *GCKR*; Table 4). The lead SNP in *GCKR* (rs1260326), a non-synonymous coding variant, was significantly associated with gene

expression of the neighboring *IFT172* gene. Further, the eSNP data supports *SLC7A9* as the important gene at the chromosome 19 susceptibility locus, as well as *ALMS1* at the susceptibility locus on chromosome 2p13, since an eSNP in perfect LD (r^2=1) with rs13538 in the HapMap CEU population is significantly associated with *ALMS1* transcript expression in lymphocytes. All eSNPs with significant association with at least one renal trait are listed in Supplementary Table 4.

Secondary Analyses: False Discovery Rate (FDR)

In order to further identify genomic loci for kidney function and disease, secondary analyses were conducted to identify SNPs that did not reach genome-wide significance but were associated with eGFRcrea or CKD at an FDR of 0.05 (p-value $<4.8*10^{-6}$). After exclusion of all SNPs within 1 Mb of a genome-wide significant SNP, 9 additional loci for eGFRcrea were identified, 4 of which were also associated with eGFRcys (Supplementary Table 5). We also compared the identified FDR-loci with the eSNP analysis; 3 regions of overlap were identified. The r^2 between each FDR SNP and the eSNP in highest LD with that FDR SNP ranged from 0.18 (*ARL15*) to 1.0 (*CASP9*, *CRKRS*), further supporting these genomic regions as loci of interest. Based on known biology, *PARD3B* and *CASP9* are particularly interesting genes emerging from FDR analyses. PARD3B is important in establishing cell polarity and localizes to tight junctions of epithelial cells;[14] it is most expressed in fetal and adult kidney. CASP9 is involved in the growth of metanephroi in the developing kidney.[15]

Discussion

Our principal findings are four-fold. We have identified 20 novel replicated loci in association with eGFR and CKD. Of these, 13 are likely to be involved in renal function and susceptibility to CKD, whereas 7 likely represent creatinine production or secretion loci. In aggregate, the 13 new renal function loci plus the three previously identified renal function loci account for 1.4% of the variation in eGFRcrea. We demonstrate altered transcript expression with SNPs at several of the identified loci, providing potential functional insight. Lastly, we provide suggestive evidence for an additional 9 eGFRcrea-associated loci using a false discovery rate metric, of which 4 loci are suspected to be related to renal function.

These findings extend previous knowledge of common genetic variation related to renal function indices. We have confirmed our prior findings, the identification of common risk variants at the *UMOD*, *SHROOM3*, and *STC1* loci as well as at two positive control loci (*GATM*, *CST*).[10] We now highlight 13 novel loci not previously known to be associated with renal function in population-based studies. In the course of our work, we have also uncovered 7 loci likely influencing creatinine production or secretion. This underscores the importance of separating genetic loci that affect concentrations of a biomarker independent of underlying disease processes from those that truly reflect disease-association. Similar to what we have previously reported,[10] we identified many more robust associations for eGFRcrea as compared to CKD. Nonetheless, nominal associations with CKD were identified for most of the renal function SNPs, showing that genetic variants associated with normal variation in eGFRcrea are also associated with the clinically important entity CKD.

Our findings highlight in several important ways how GWAS can aid in uncovering the genetic underpinnings of complex human traits and diseases as well as represent a first step towards a better understanding of physiological mechanisms and pathways. First, they provide novel information about the allelic architecture of known risk loci for genetic diseases of the kidney. Rare mutations in *SLC7A9*, *SLC34A1*, *ALMS1*, and *UMOD* are known to cause monogenetic diseases that feature a renal phenotype, underscoring the additional importance of common genetic susceptibility variants in these genes. This phenomenon is also observed for other complex traits and diseases; approximately 20% of

loci discovered in GWAS of a variety of complex traits are known to also harbor mutations that cause monogenic diseases.[16]

Second, our findings provide information about the genomic location of genetic variants associated with renal indices. Over 65% of the SNPs identified in our discovery analyses are located in or within 3.7 kb upstream of genes, and three of the variants are non-synonymous coding. This is in agreement with a recent study that reported an enrichment of trait-associated variants identified in GWAS at non-synonymous coding sites and a depletion of trait-associated variants in intergenic regions when compared to a random selection of variants on genotyping arrays.[17;18]

Third, our replicated findings highlight the role of several pathways and mechanisms of importance in renal development and function. We identified common genetic variants in genes related to nephrogenesis (*ALMS1*, *VEGFA*, potentially *DACH1*), glomerular filtration barrier formation and podocyte function (*DAB2*, *PARD3B*, *VEGFA*), angiogenesis (*VEGFA*), solute transport (*SLC7A9*, *SLC34A1*), and metabolic functions of the kidney (*PRKAG2*, potentially *GCKR* and *LASS2*). Several of the genes we identified can be linked to the role of primary cilia (*ALMS1*, *GCKR*/*IFT172*, *PARD3B*); mutations in genes with a role in development and function of primary cilia are known to cause hereditary genetic diseases of the kidney such as polycystic kidney disease and nephronophthisis.[19] The ability to uncover genetic variation in these genes in unselected individuals from population-based studies emphasizes their contribution to important mechanisms related to renal function in humans under physiological conditions and should provide interesting candidates for follow up in functional studies.

Finally, our data provide novel information about components of creatinine metabolism in humans and specifically about how creatinine is handled by epithelial cells of the proximal renal tubule. This may be of interest not only to physiologists but may also have consequences on the precision of GFR estimation from serum creatinine in the clinical setting.

Similar to what has been observed previously, we identified modest effects of the risk alleles on eGFR and CKD. Taken together, these renal function loci are associated with 1.4% of the variation in eGFRcrea. We observed substantial gradation of CKD prevalence across the genetic risk score, indicating the potential clinical relevance of these risk alleles. Most importantly, our findings point toward novel mechanisms that may lead to a better understanding of both renal development and the pathogenesis of CKD.

The strength of our analysis lies in the large sample size of 67,093 used for discovery, allowing us to uncover multiple loci despite the small effect size on eGFR and CKD. We restricted our analyses to population-based studies thereby avoiding potential bias from using case-control samples[20] or from potential counter-regulating disease processes. We employed several additional methods to enhance our ability to discover novel kidney disease susceptibility loci in this screen free of prior biological hypotheses including an FDR and eSNP approach. To enable discrimination of renal function loci from creatinine production and secretion loci, we used a separate complementary measure of glomerular filtration obtained from cystatin C.

Some limitations warrant mention. Our sample consists of white participants only, and it is uncertain whether these results would replicate in other ethnic groups. We used an indirect measure of GFR as estimated by the MDRD equation; gold-standard measures of glomerular filtration are not feasible in a large population-based setting. We used eGFRcys to provide confirmatory evidence of which novel loci were indeed renal function loci. Loci that were not associated with eGFRcys were designated as presumptive creatinine production or

secretion loci, but the smaller sample size with available cystatin C measures limits the power to confirm renal function loci. We may have falsely labeled some loci as unrelated to renal function based on an absent association with eGFRcys, particularly *WDR37* and *WDR72*, which showed association of borderline significance with eGFRcys. Lastly, several genes exist in the regions of interest. For many of the reported loci, we are unable to identify which is the most likely gene related to the SNP association based on statistical evidence, although we could address this question to some extent using the eSNP data.

Multiple common genetic variants are associated with indices of renal function and highlight the role of specific genes in nephrogenesis, podocyte function, angiogenesis, solute transport, and metabolic functions of the kidney.

Box 1. Genes of special interest at newly discovered susceptibility loci for kidney function and disease

SLC7A9

SLC7A9 encodes for an amino acid transporter in renal proximal tubule cells; mutations in *SLC7A9* cause cystinuria type B (OMIM #220100).[21] Patients with cystinuria excrete elevated amounts of amino acids, resulting in the formation of stones in the urinary tract. *SLC7A9*-deficient mice display tubular and pelvic dilatation, tubular necrosis, and chronic interstitial nephritis.[22]

SLC34A1

Mutations in *SLC34A1* cause hypophosphatemic nephrolithiasis/osteoporosis (OMIM #612286).[23] *SLC34A1* encodes the type IIa Na/Pi cotransporter, which is exclusively expressed in kidney and located in the brush border of renal proximal tubular cells, where it mediates reuptake of inorganic phosphate.[24]

ALMS1

Mutations in *ALMS1* cause the autosomal recessive Almstrom Syndrome (OMIM #203800), characterized by retinal degeneration, hearing loss, obesity, diabetes, and commonly renal insufficiency.[25;26] Mutations in this gene are associated with age-dependent ciliopathies in the kidney.27

DAB2

DAB2 is a cytoplasmatic adaptor protein expressed in renal proximal tubular cells,[28] where it is reported to represent the physical link between megalin and non-muscle myosin heavy polypeptide 9, encoded by *MYH9*.[29] Common variants in *MYH9* were recently identified as important susceptibility alleles for non-diabetic kidney disease in African Americans.[30;31]

VEGFA

Encodes for vascular endothelial growth factor A, with roles in angiogenesis and vascular permeability.[32] Renal podocytes produce large amounts of VEGFA, which is essential for glomerulogenesis and glomerular filtration barrier formation in animal models.[32] *VEGFA* has been reported to affect ureteric bud growth during embryogenesis and hence may impact the number of nephrons.[33]

GCKR

The product of *GCKR* inhibits hepatic glucokinase.[34] Common variants in *GCKR*, and specifically the missense SNP rs1260326 (P446L), are associated with a variety of human traits in genetic association studies, including serum triglycerides, fasting glucose, C-reactive protein, and uric acid as well as susceptibility to type 2 diabetes

(http://www.genome.gov/GWAstudies/), highlighting the pleiotropy of this locus. eSNP analyses point to the role of the neighboring *IFT172* gene, which has a role in the formation of primary cilia.[35]

PRKAG2

Rare *PRKAG2* variants cause a form of heart disease, featuring hypertrophic cardiomyopathy and the Wolff-Parkinson-White syndrome[36;37] and sometimes enlarged kidneys.[38] Studies in transgenic mice indicate that these mutations cause a glycogen storage disease of the heart.[37] Several other hereditary glycogen storage diseases present with renal pathology such as renal tubular dysfunction.[39]

DACH1

Dachshund homologue 1 is a transcription factor with a role in organogenesis; our data implicate a 100kb region within this gene. It is expressed in adult human kidney, as well as murine developing kidney, specifically glomerular podocytes and tubular epithelial cells.[40] *DACH1* may have a role in the development of the Mullerian duct.[41] *DACH1* is part of the genetic network including *SIX* and *EYA*,[42] mutations in which cause brachio-oto-renal syndrome.[43]

LASS2

is highly expressed in the kidney and may be involved in cell growth.[44] A non-synonymous coding SNP in *LASS2*, rs267738 (E115A), was in perfect LD with the lead SNP in the region and of predicted damaging function.[45] *LASS2* has been implicated in the synthesis of specific ceramides.[46] Ceramides and their product sphingolipids are important in genetic diseases of the kidney,[47] and have a role in aging mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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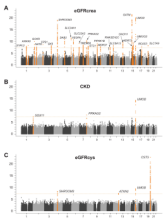

Figure 1.
Genome-wide $-\log_{10}$ p-value plot from Stage 1: Discovery analysis of eGFRcrea (**A**), CKD (**B**) and eGFRcys (**C**).

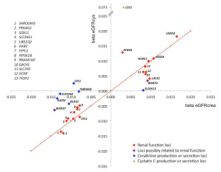

Figure 2.

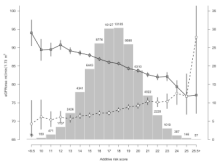

Figure 3.

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Table 1

Study Sample Characteristics in Stage 1 Discovery and Stage 2 Replication samples, CKDGen Consortium

Study	Sample Size eGFRcrea/CKD/eGFRcys	Women %	Age (years)	eGFRcrea (ml/min/1.73 m^2)	eGFRcys (ml/min/1.73 m^2)	CKD* %
Stage 1: Discovery						
AGES	3219/3219/NA	58.0	76.4 (5.5)	73.0 (20.0)	NA	24.3
Amish	1211/NA/783	49.0	49.3 (16.8)	93.8 (19.6)	114.9 (17.9)	2.9
ARIC	8069/8069/6430	53.0	62.0 (6.1)	81.3 (17.7)	84.1 (19.7)	9.1
ASPS	850/850/NA	56.8	65.2 (8.1)	96.5 (39.9)	NA	8.1
BLSA	723/723/NA	46.1	70.4 (15.2)	80.3 (23.1)	NA	17.4
CHS	3259/3259/2820	53.0	72.3 (5.4)	80.0 (22.6)	79.9 (18.3)	18.5
ERF	2079/2079/NA	56.3	49.2 (14.0)	93.5 (21.4)	NA	3.8
Family Heart Study	883/883/NA	51.1	55.5 (11.1)	88.5 (19.4)	NA	4.4
Framingham Heart Study	7782/4140/2992	54.3	51.2 (14.0)	92.1(21.7)	77.9(16.9)	10.7
KORA F3	1641/1641/1642	50.5	62.5 (10.1)	78.7 (19.0)	111.9 (26.6)	10.7
KORA F4	1814/1814/1811	51.3	60.9 (8.9)	85.1 (20.2)	109.7 (26.2)	7.0
Korcula	888/888/NA	64.0	56.3 (13.9)	87.3 (20.6)	NA	7.5
Micros	1201/1201/1248	56.8	46.2 (16.1)	94.6 (20.9)	107.4 (23.6)	3.8
ORCADES	704/704/NA	53.6	54.1 (15.2)	89.4 (20.7)	NA	6.8
Rotterdam Study I	4390/4390/NA	61.4	70.0 (9.0)	77.1 (17.2)	NA	13.7
Rotterdam Study II	1863/1863/NA	54.5	64.8 (8.0)	81.3 (17.2)	NA	9.1
NHSPS	565/565/NA	53.1	51.7 (18.3)	90.9 (22.1)	NA	5.7
SHIP	3231/3228/3231	51.7	54.5 (15.3)	90.5 (23.6)	97.1 (25.31)	7.7
Vis	768/768/NA	58.6	56.9 (15.2)	88.1 (22.1)	NA	6.9
WGHS	21953/21953/NA	100.0	54.7 (7.1)	90.3 (22.5)	NA	6.1
Total	67093/62237/20957					
Stage 2: Replication						
ARIC	944/944/751	54.8	61.9 (6.13)	81.6 (16.2)	85.4 (19.6)	7.5
GENOA	1056/1056/NA	56.2	59.0 (10.2)	85.2 (22.8)	NA	12.0
Family Heart Study	1537/1537/NA	57.1	47.8 (13.3)	93.7 (19.7)	NA	2.2
Gutenberg Heart Study	3180/3180/NA	48.7	55.9 (10.9)	87.1 (16.6)	NA	3.8

Study	Sample Size eGFRcrea/CKD/eGFRcys	Women %	Age (years)	eGFRcrea (ml/min/1.73 m^2)	eGFRcys (ml/min/1.73 m^2)	CKD* %
Stage 1: Discovery						
Health ABC	1663/1663/1663	47.2	73.7(2.9)	70.1 (14.5)	77.3 (20.1)	25.1
HPFS	818/818/NA	0	64.7 (8.3)	85.2 (22.7)	NA	9.5
KORA F3	1498/1498/1498	52.5	51.6 (13.3)	95.2 (24.2)	123.5 (29.5)	4.0
KORA F4	1202/1202/1202	52.3	49.2 (15.4)	92.9 (23.2)	118.4 (27.5)	5.9
Nurses Health Study	786/786/NA	100.0	59.5 (6.5)	86.2 (22.1)	NA	10.7
POPGEN	1163/1163/NA	40.6	53.8 (14.4)	88.1 (18.8)	NA	5.1
SAPALDIA	6031/6031/NA	50.2	52.2 (11.4)	90.7 (17.3)	NA	2.9
SAPHIR	1733/1733/NA	37.0	51.4 (6.0)	91.7 (16.0)	NA	1.1
SORBS	892/892/NA	58.3	48.5 (15.8)	92.6 (19.3)	NA	4.1
SPLIT	479/NA/NA	58.9	49.2 (14.5)	95.59 (24.1)	NA	3.5
Total	22982/22503/5114					

* Total of 5807 CKD cases in the Stage 1 Discovery sample and 1366 CKD cases in Stage 2 Replication.

Abbreviations: eGFRcrea: estimated glomerular filtration rate by serum creatinine, eGFRcys: estimated glomerular filtration rate by serum cystatin C, CKD: chronic kidney disease, NA: not available.

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Table 2

Genome-wide Significant Loci: SNP Association with Renal Traits in Stage 1 Discovery And Stage 2 Replication Meta-Analyses

Trait	SNP ID	Chr	position (b36)	Genes Within 60 kb	SNP function	Minor Allele Freq[#]	Trait Discovery P-value[*]	Trait Replication P-value	Trait Meta-analysis P-value	eGFRcys P-value[§]
				Known Loci						
eGFRcrea[*]	rs17319721	4	77587871	**SHROOM3**;FLJ25770	intronic	0.43	1.1E-19	NA	NA	NA
eGFRcrea	rs10109414	8	23807096	STC1	intergenic	0.42	1.0E-08	NA	NA	NA
eGFRcrea	rs2453533	15	43428517	GATM;SPATA5L1	intergenic	0.38	4.6E-22	NA	NA	NA
eGFRcrea[*]	rs12917707	16	20275191	UMOD;FLJ20581,GP2,PDILT	upstream	0.18	1.2E-20	NA	NA	NA
eGFRcys	rs911119	20	23560737	CST3;CST4,CST9	intergenic	0.21	2.3E-138	NA	NA	NA
				Novel Renal Function Loci						
eGFRcrea	rs1933182	1	109801361	SYPL2;ATXN7L2,CYB561D1,PSMA5,AMIGO1,SORT1	intergenic	0.30	1.3E-08	1.9E-01	**1.2E-07**	5.0E-02
eGFRcrea	rs267734	1	149218101	ANXA9;FAM63A,PRUNE,BNIPL,LASS2,SETDB1	upstream	0.20	5.2E-09	1.1E-04	**1.2E-12**	9.7E-03
CKD	rs16864170	2	5825331	SOX11	intergenic	0.05	4.5E-08	3.5E-01	**1.6E-07**	2.9E-02
eGFRcrea	rs1260326	2	27584444	**GCKR**;IFT172,FNDC4	non-synonymous	0.41	1.3E-10	1.1E-04	**3.0E-14**	3.7E-03
eGFRcrea	rs13538	2	73721836	**NAT8**;NAT8B,ALMS1	non-synonymous	0.23	2.6E-08	7.2E-07	**4.5E-14**	1.6E-04
eGFRcrea	rs347685	3	143289827	TFDP2	unknown	0.28	7.0E-09	1.4E-03	**3.0E-11**	1.2E-03
eGFRcrea	rs11959928	5	39432889	**DAB2**;C9	intronic	0.44	1.8E-11	5.6E-07	**1.4E-17**	1.6E-04
eGFRcrea	rs6420094	5	176750242	**SLC34A1**;GRK6,RGS14,LMAN2,PRR7,F12,PFN3	intronic	0.34	3.8E-12	6.6E-04	**1.0E-14**	1.3E-05
eGFRcrea	rs881858	6	43914587	VEGFA	intergenic	0.28	2.2E-11	7.7E-04	**9.4E-14**	3.7E-03
CKD[*]	rs7805747	7	151038734	**PRKAG2**	intronic	0.24	8.6E-09	7.7E-05	**4.2E-12**	8.1E-07
eGFRcrea	rs4744712	9	70624527	**PIP5K1B**;FAM122A	intronic	0.39	7.2E-10	6.6E-05	**8.3E-14**	6.8E-03
eGFRcrea	rs4014195	11	65263398	RNASEH2C;DKFZp761E19 8,HTATIP,OVOL1	intergenic	0.35	3.3E-08	1.4E-01	**1.4E-07**	2.7E-02
eGFRcys	rs653178	12	110492139	**ATXN2**	intronic	0.50	3.8E-08	1.4E-04	**3.5E-11**	3.5E-11
eGFRcrea	rs626277	13	71245697	**DACH1**	intronic	0.40	2.9E-10	1.0E-02	**2.5E-11**	6.5E-04
eGFRcrea	rs1394125	15	73946038	**UBE2Q2**;FBXO22	intronic	0.35	3.7E-10	4.7E-08	**3.3E-17**	6.9E-04
eGFRcrea	rs12460876	19	38048731	**SLC7A9**;CCDC123,ECAT8	intronic	0.39	5.5E-09	2.5E-07	**3.2E-15**	1.2E-02
				Novel Creatinine Production and Secretion Loci						
eGFRcrea	rs7422339[**]	2	211248752	**CPS1**	non-synonymous	0.32	2.4E-09	2.6E-07	**1.2E-15**	1.9E-01
eGFRcrea	rs2279463	6	160588379	**SLC22A2**	intronic	0.12	8.7E-10	1.7E-03	**5.5E-12**	4.8E-01

Trait	SNP ID	Chr	position (b36)	Genes Within 60 kb	SNP function	Minor Allele Freq[#]	Trait Discovery P-value[*]	Trait Replication P-value	Trait Meta-analysis P-value	eGFRcys P-value[§]
eGFRcrea	rs6465825	7	77254375	TMEM60;RSBN1L,PHTF2	intergenic	0.39	3.5E-09	3.5E-02	**1.5E-09**	7.4E-01
eGFRcrea	rs10794720	10	1146165	**WDR37**	intronic	0.08	2.1E-08	4.7E-02	**1.2E-08**	8.9E-02
eGFRcrea	rs10774021	12	219559	**SLC6A13**;JARID1A,SLC6A 12	intronic	0.36	6.7E-09	7.1E-02	**1.4E-09**	7.9E-01
eGFRcrea	rs491567	15	51733885	**WDR72**	intronic	0.22	1.3E-08	1.0E-05	**2.7E-13**	8.1E-02
eGFRcrea	rs9895661	17	56811371	**BCAS3**;TBX2,C17orf82	intronic	0.19	1.4E-08	3.0E-08	**1.1E-15**	2.8E-01

[*] Only rs12917707 at the UMOD locus demonstrated significant heterogeneity.

[#] The minor allele based on sample size weighted mean allele frequency in the discovery cohorts is modeled.

[*] SNP also significantly associated with at least one other kidney trait at p<5×10E-08.

[**] SNP rs7422339 has changed the SNP identifier in the latest dbSNP release,current name is rs1047891.

[§] Results of eGFRcys from Discovery and Replication combined. For *ATXN2*, the p-value for eGFRcrea from Stage 1 + Stage 2 is 0.0005. NA: known loci were not replicated in additional study samples. Genes within 60 kb were based on RefSeq genes (b36). The gene closest to the SNP is listed first and bold if the SNP is located within the gene. Other genes in the region are listed after ";".

Abbreviations: Chr=chromosome.

Table 3

Association of Replicated Renal Function Susceptibility Loci with CKD

	Chr	position (b36)	Genes Nearby	modeled allele[#]	OR CKD [§]	95% CI	p-value
rs267734	1	149218101	ANXA9;FAM63A,PRUNE,BNIPL,LASS2,SETDB1	c	0.93	0.88–0.97	2.6E-03
rs1260326	2	27584444	**GCKR**;IFT172,FNDC4	t	0.96	0.93–1.00	8.7E-02
rs13538	2	73721836	**NAT8**;NAT8B,ALMS1	g	0.93	0.89–0.98	6.3E-03
rs347685	3	143289827	TFDP2	c	0.93	0.89–0.97	1.2E-03
rs11959928	5	39432889	**DAB2**;C9	a	1.08	1.04–1.12	1.5E-05
rs6420094	5	176750242	**SLC34A1**;GRK6,RGS14,LMAN2,PRR7,F12,PFN3	g	1.08	1.03–1.12	2.1E-04
rs881858	6	43914587	VEGFA	g	0.93	0.89–0.97	3.7E-03
rs7805747	7	151038734	**PRKAG2**	a	1.19	1.13–1.25	4.2E-12
rs4744712	9	70624527	**PIP5K1B**;FAM122A	a	1.06	1.02–1.10	7.0E-04
rs653178	12	110492139	**ATXN2**	t	0.96	0.92–1.00	5.8E-02
rs626277	13	71245697	**DACH1**	c	0.94	0.91–0.98	4.7E-03
rs1394125	15	73946038	**UBE2Q2**;FBXO22	a	1.08	1.04–1.13	8.0E-05
rs12460876	19	38048731	**SLC7A9**;CCDC123,ECAT8	c	0.93	0.89–0.96	2.6E-04

[#]The minor allele based on sample size weighted mean allele frequency in the discovery cohorts is modeled.

[§]Results based on Stage 1: Discovery and Stage 2: Replication combined. Genes within 60 kb were based on RefSeq genes. The gene closest to the SNP is listed first and bold if the SNP is located within the gene. Other genes in the region are listed after ";". P-values from the discovery screen are corrected for genomic control before and after meta-analysis.

Abbreviations: OR: odds ratio, CKD: chronic kidney disease, Chr=chromosome.

Table 4

Significant eSNPs associated with eGFRcrea, CKD, or eGFRcys

P-values in bold are <1/n, with n being the number of eSNPs queried (3322 for liver, 10823 for lymphpblastoid cell lines (LCL), and 29094 for lymphocytes).

SNP	Tissue	chr	Genes within 60kb of SNP	p-value eSNP	expressed gene	p-value eGFRcys	p-value CKD	p-value eGFRcrea	r2 to top SNP	top SNP
rs1260326	lymphocytes, LCL*	2	GCKR;IFT172, FNDC4	7.0E-12	IFT172#	6.4E-03	1.8E-01	**1.3E-10**	identical	rs1260326
rs10198549	lymphocytes, liver*	2	ALMS1	4.8E-09	ALMS1#	9.1E-04	1.4E-01	**3.8E-07**	1	rs13538
rs6440052	lymphocytes	3	TFDP2;ATP1B3	5.2E-22	ATP1B3	1.5E-02	3.0E-04	**5.0E-07**	0.67	rs347685
rs4256249	liver	4	SHROOM3	4.3E-06	SHROOM3	2.7E-01	8.0E-02	**1.0E-05**	0.06	rs17319721
rs835223	lymphocytes	5	DAB2;C9	4.7E-04	DAB2	5.5E-03	**2.2E-05**	**1.7E-10**	0.93	rs11959928
rs1544457	LCL	7	TMEM60;RSBN1L,PHTF2	2.9E-08	PTPN12#	2.9E-01	1.8E-01	**4.4E-07**	0.72	rs6465825
rs409783	liver	8	STC1	5.5E-07	C2orf29	1.3E-02	4.2E-01	**3.1E-04**	0.29	rs10109414
rs7035163	liver	9	FAM122A;PIP5K1B	2.4E-11	FAM122A	8.0E-01	2.4E-02	**4.0E-05**	0.19	rs4744712
rs11062357	lymphocytes	12	JARID1A;SLC6A13	5.5E-07	JARID1A	5.3E-01	1.9E-01	**2.4E-06**	0.37	rs10774021
rs335675	lymphocytes, liver*	15	FBXO22;NRG4, UBE2Q2	2.1E-03	FBXO22#	2.0E-04	4.3E-01	**9.4E-08**	0.01	rs1394125
rs16967572	liver	19	CCDC123;RHPN2, SLC7A9,C19orf40	9.6E-13	SLC7A9	5.3E-02	1.9E-01	**7.2E-05**	0.58	rs12460876

*Other significant eSNPs in the same region were significantly associated with kidney traits in the second tissue listed. The SNPs, expressed genes, and the r^2 to the lead SNP in the region can be found in Supplementary Table 6.

#Other significant eSNPs in the extended gene region were significantly associated with eGFRcrea and expression of other genes nearby. R^2 to the lead SNP in the region was lower. The SNPs, expressed genes, and r^2 to the lead SNP are listed in Supplementary Table 6. Additionally, eSNPs in creatinine and cystatin production loci GATM and CST3 were significanly associated with eGFRcrea and eGFRcys, respectively, in lymphocytes and are listed in Supplementary Table 4. Genes within 60 kb were based on RefSeq genes. The gene closest to the SNP is listed first and separated by other genes in the region by ";".